

07-19-00

A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Ambikaipakan Balasubramaniam, William T.
Chance

Atty. Docket: UOC/136R

Title: Compounds for Control of Appetite, Blood Pressure, Cardiovascular Response, Libido and Circadian Rhythm

CERTIFICATE OF MAILING BY EXPRESS MAIL - 37 CFR 1.10'Express Mail' mailing label number: EL420322439USDate of Deposit: July 18, 2000

I certify that this paper or fee (along with the enclosures noted herein) is being deposited with the United States Postal Service 'Express Mail Post Office to Addressee' service under 37 CFR 1.10 on the above date and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Maria C. Rodriguez
By: Maria C. Rodriguez (person mailing paper)

UTILITY PATENT APPLICATION TRANSMITTAL**BOX PATENT APPLICATION**

Assistant Commissioner for Patents
Washington, D.C. 20231

This is a request for filing, under 37 CFR § 1.53(b), a(n):

- ☐ Original (non-provisional) application.
- ☐ Divisional of prior application Serial No. __, filed on __.
- ☐ Continuation of prior application Serial No. __, filed on __.
- ☒ **Continuation-in-part of prior application Serial No. 09/449,914, filed on December 2, 1999.**

For Divisional and Continuation applications ONLY:

- ☐ Copy of Executed Declaration, Power of Attorney and Petition Form from parent application is enclosed (as noted below in Enclosures).
- ☐ Incorporation by Reference: The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied herewith, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

For all Divisional, Continuation and Continuation-in-part applications:

- ☒ **A Small Entity Statement was filed in the prior application, small entity status is still proper and desired.**
- ☐ This application is assigned to __ by virtue of an earlier assignment filed in the prior application at Reel __, Frame __.

PRELIMINARY AMENDMENT/CALCULATION OF FEES

- ☐ Please cancel claims ___ without prejudice, and prior to calculating the fees. ___ total claim(s), of which ___ is(are) independent, is(are) pending after the amendment.
- ☐ Please enter the enclosed preliminary amendment identified below prior to calculating the fees. ___ total claim(s), of which ___ is(are) independent, is(are) pending after the amendment.
- ☒ **The Fees are Calculated as Follows:**

Fee:	Number of Claims:	In Excess of:	Extra:	At Rate:	Amount:
Total Claims	39	20	19	\$18	\$342.00
Independent Claims	3	3	0	\$78	\$0.00
MULTIPLE DEPENDENT CLAIM FEE					
BASIC FEE					\$690.00
TOTAL OF ABOVE CALCULATIONS					\$1,032.00
REDUCTION BY 50% FOR FILING BY SMALL ENTITY					\$516.00
TOTAL					\$516.00

ENCLOSURES

- ☒ **Utility Patent Application Transmittal Form (in duplicate) containing Certificate of Mailing By Express Mail Under 37 CFR 1.10.**
- ☒ **Return Postcard.**

APPLICATION PAPERS

- ☒ **Utility Patent Application, with: cover sheet, 57 page(s) specification (including 39 total claim(s), of which 3 is(are) independent), and 1 page(s) abstract.**
- ☒ **Drawings: 3 sheet(s) of formal drawings (3 total figure(s)).**
- ☐ Microfiche Computer Program (Appendix).
- ☐ Nucleotide and/or Amino Acid Sequence, including (all are necessary): Computer Readable Copy, Paper Copy (identical to computer copy), and Statement verifying identity of copies.
- ☒ **An Executed Declaration, Power of Attorney and Petition Form.**
- ☐ Copy of Executed Declaration, Power of Attorney and Petition Form from prior application identified above.
- ☐ Certified Copy of priority document(s) identified as attached above.

ADDITIONAL PAPERS

- ☒ **Assignment to University of Cincinnati, Recordation Cover Sheet (Form PTO-1595)**
- ☐ Verified Statement to Establish Small Entity Status under 37 CFR 1.9 and 1.27.
- ☐ Preliminary Amendment (to be entered prior to calculation of fees)
- ☐ Information Disclosure Statement, ___ sheet(s) Form PTO-1449, ___ U.S. Patent Reference(s), ___ Foreign Patent Reference(s) and ___ Other Reference(s)
- ☐ Other: ___

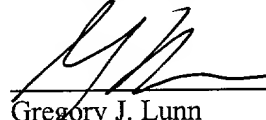
CHECKS

- ☒ **A Check of \$516.00 for the filing fee.**
- ☒ **A Check of \$40.00 for the assignment recording fee.**

DEPOSIT ACCOUNT AUTHORIZATION

- ☐ Please charge Deposit Account No. 23-3000 in the amount of .
- ☒ **The Commissioner is authorized to charge any fees under 37 CFR 1.16 and 1.17 which may be required during the entire pendency of the application, or credit any overpayment, to Deposit Account No. 23-3000. A duplicate of this transmittal is attached.**
- ☐ THE PAYMENT OF FEES IS BEING DEFERRED.
-

Respectfully Submitted,



Gregory J. Lunn
Reg. No. P-29,945

WOOD, HERRON & EVANS, L.L.P.
2700 Carew Tower
Cincinnati, Ohio 45202
(513) 241-2324

GJL/eam

PATENT

Applicant or Patentee: Ambikaipakan **BALASUBRAMANIAM** and

William T. CHANCE

Attorney's Docket No.: UOC 113A 111

Serial or Patent No.:

Filed or Issued:

For:

**Compounds for Control of Appetite, Blood Pressure,
Cardiovascular Response, Libido, and Circadian Rhythm**

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY
STATUS (37 CFR 1.9(f) and 1.27(d)) - NONPROFIT ORGANIZATION**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION:

UNIVERSITY OF CINCINNATI

ADDRESS OF CONCERN:

2600 Clifton Avenue

Cincinnati, Ohio 45221-0623

TYPE OF ORGANIZATION:

- ☒ **UNIVERSITY.** **University of Cincinnati**
- ☒ **TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. 501(a) AND 501(c)(3)).**
- ☒ **NONPROFIT SCIENTIFIC OR EDUCATIONAL INSTITUTION UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA. (Name of State: **OHIO**)**
- ☐ **WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. 501(a) AND 501(c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA**
- ☐ **WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA (Name of State: _____) (Citation of Statute: _____)**

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. 1.9(e) for purposes of paying reduced fees under §§ 41 (a) and (b) of Title 35, United States Code, with regard to the invention entitled **Compounds for Control of Appetite, Blood Pressure, Cardiovascularby inventors Ambikaipakan BALASUBRAMANIAM and William T. CHANCE** described in:

- ☒ the specification filed herewith
- ☐ application serial number _____, filed _____
- ☐ patent no. _____, issued _____

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. 1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(d) or a nonprofit organization under 37 C.F.R. 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities.
(37 C.F.R. 1.27)

NAME _____

ADDRESS _____

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in the application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING

TITLE OF PERSON OTHER THAN OWNER

ADDRESS OF PERSON SIGNING

Stephen R. Albainy-Jenei

Assistant General Counsel and
Assistant University Patent Officer

2600 Clifton Avenue
Cincinnati, Ohio 45221-0623

Date: 7 August 97

Stephen R. Albainy-Jenei
SIGNATURE

Stephen R. Albainy-Jenei, J.D.
Assistant General Counsel and
Assistant University Patent Officer
University of Cincinnati

Express Mail No. EL420322439US

APPLICATION FOR UNITED STATES PATENT

Applicant(s): Ambikaipakan Balasubramaniam and
William T. Chance

Title: COMPOUNDS FOR CONTROL OF APPETITE, BLOOD
PRESSURE, CARDIOVASCULAR RESPONSE,
LIBIDO, AND CIRCADIAN RHYTHM

Assignee: University of Cincinnati

Wood, Herron & Evans, L.L.P.
2700 Carew Tower
Cincinnati, Ohio 45202
Attorneys
(513) 241-2324 (voice)
(513) 421-7269 (fax)
Our Ref.: UOC-136R

SPECIFICATION

**COMPOUNDS FOR CONTROL OF APPETITE, BLOOD PRESSURE,
CARDIOVASCULAR RESPONSE, LIBIDO, AND CIRCADIAN RHYTHM**

5

**Ambikaipakan Balasubramaniam
William T. Chance**

10

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant/Contract No. GM47122 awarded by the National Institutes of Health.

15

This application is a continuation-in-part of U.S. Application Serial No. 09/449,914, filed on December 2, 1999, which is a divisional of U.S. Patent No. 6,013,633, issued January 11, 2000.

Background of the Invention

20

This invention relates generally to dipeptides and tripeptides and to methods for pharmaceutical treatment of mammals using analogs of such dipeptides and tripeptides. More specifically, the invention relates to tripeptides and their analogs, to pharmaceutical compositions containing such dipeptides and tripeptides and to methods of treatment of mammals using such dipeptides and tripeptides. In addition, the invention relates to methods of treatment of mammals using such dipeptides and tripeptides for control of appetite, blood pressure, cardiovascular response, libido, and circadian rhythm.

25

1. Appetite and obesity

Obesity is a major disorder affecting as much as one third of the North American population. Several studies have shown that such individuals are at increased risk in developing cardiovascular disease (hypertension and

5 hypercholesterolemia), diabetes and several types of cancer. The effective treatment of obesity, however, remains a largely unachieved goal. Existing pharmacotherapeutic approaches to weight loss involve the use of amphetamine-based agents such as amphetamine, diethylpropion, mazindol and fenfluramine which act directly on the CNS to lower food intake by modulating dopaminergic, adrenergic
10 and/or serotonergic mechanisms. Although weight loss can be achieved with such agents, their use is restricted due to CNS side-effects, potential addiction liability and the production of tolerance to their actions, with chronic administration leading to potential depression, vestibular disturbances, hallucinations and addiction, as well as interference with the actions other drugs such as MAO inhibitors and
15 antihypertensives. There is also a subpopulation of obese patients that is refractory to present anorectic drug treatments. The medical need is high for an effective anorectic agent which overcomes the above disadvantages of existing therapies. Of particular need are agents which act by alternative mechanisms to modulate food intake and/or metabolism.

20 2. Neuropeptide Y ("NPY")

Throughout this application, various publications are referenced. The disclosure of these publications is hereby incorporated by reference into this application to describe more fully the art to which this invention pertains.

Neuropeptides are small peptides originating from large precursor proteins synthesized by peptidergic neurons and endocrine/paracrine cells. They hold promise for treatment of neurological, psychiatric, and endocrine disorders (De Wied, D. In: *Neuropeptides: Basics and Perspectives* (Elsevier, Amsterdam-New York-Oxford), 1990.). Often the precursors contain multiple biologically active peptides. There is great diversity of neuropeptides in the brain caused by alternative splicing of primary gene transcripts and differential precursor processing. The neuropeptide receptors serve to discriminate between ligands and to activate the appropriate signals. Thus, it is expected that the receptors for neuropeptides consist of a large number of members.

Neuropeptide Y (NPY), a 36-amino acid peptide, is the most abundant neuropeptide to be identified in mammalian brain. NPY is an important regulator in both the central and peripheral nervous systems (Heilig, M. and E. Widerlov. *Neuropeptide Y: an overview of central distribution, functional aspects, and possible involvement of neuropsychiatric illnesses. Acta Psychiatr. Scand.* 82:95-114 (1990).) and influences a diverse range of physiological parameters, including effects on psychomotor activity, food intake, central endocrine secretion, and vasoactivity in the cardiovascular system. High concentrations of NPY are found in the sympathetic nerves supplying the coronary, cerebral, and renal vasculature and has contributed to vasoconstriction. NPY binding sites have been identified in a variety of tissues, including spleen (Lundberg, J. M., A. Hemsén, O. Larsson, A. Rudehill, A. Saria, and B. Fredholm. *Neuropeptide Y receptor in pig spleen: binding characteristics, reduction of cyclic AMP formation and calcium antagonist inhibition of*

vasoconstriction. Eur. J. Pharmacol. 145:21-29 (1988)), intestinal membranes, brain (Hinson, J., C. Rauh, and J. Coupet. Neuropeptide Y stimulates inositol phospholipid hydrolysis in rat brain microprisms. Brain RESPONSE. 446:379-382 (1988)), aortic smooth muscle (Mihara, S., Y. Shigeri, and M. Fujimoto. Neuropeptide Y-induced intracellular Ca²⁺ increase in vascular smooth muscle cells. FEBS Lett. 259: 79-82 (1989)), kidney, testis, and placenta (Dumont, Y., J.C. Martel, A. Fournier, S. St.-Pierre, and R. Quiron. Neuropeptide Y and neuropeptide Y receptor subtypes in brain and peripheral tissues. Prog. Neurobiol. 38:125-167 (1992)). In addition, binding sites have been reported in a number of rat and human cell lines (e.g. Y1 in SK-N-MC, MC-IXC, CHP-212, and PC12 cells; Y2 in SK-N-Be(2), CHP-234, and SMS-MSN)(Grundemar, L., S. P. Sheikh, and C. Wahlestedt, In: The Biology of Neuropeptide Y and Related Peptides. (Humana Press, Inc., Totawa, New Jersey), (1992)).

NPY forms a family (called the pancreatic polypeptide family) together with pancreatic polypeptide (PP) and peptide YOSHIOKA (PYY) which all consist of 36 amino acids and have a common tertiary structure, the so-called PP-fold (Glover, I. D., D. J. Barlow, J. E. Pitts, S. P. Wood, I. J. Tickle, T. L. Blundell, K. Tatemoto, J. R. Kimmel, A. Wollmer, W. Strassburger, and Y.-S. Zhang. Conformational studies of the pancreatic polypeptide hormone family. Eur. J. Biochem. 142:379-385 (1985)). Specific features of this family include a polyproline helix in residues 1 through 8, beta -turn in residues 9 through 14, an alpha -helix in residues 15 through 30, an outward-projecting C-terminus in residues 30 through 36, and a carboxy terminal amide which appears to be critical for biological activity (Schwartz, T. W., J.

Fuhlendorff, L. L. Kjems, M. S. Kristensen, M. Vervelde, M. O'Hare, J. L.

Krstenansky, and B. Bjornholm. Signal epitopes in the three-dimensional structure of neuropeptide Y. *Ann. N.Y. Acad. Sci.* 611:35-47 (1990)). The C-terminal amidated residue of these peptides is essential for biological activity (Wahlestedt *et al.*, 1986).

- 5 Studies with peptide fragments of NPY have indicated that multiple NPY receptor subtypes exist (Wahlestedt, C., N. Yanaihara, and R. Hakanson. Evidence for different pre- and postjunctional receptors for neuropeptide Y and related peptides. *Regul. Pept.* 13:307-318 (1986)). Three major NPY receptor subtypes (Y1, Y2 and Y3) have been defined by pharmacological criteria, with a fourth "atypical" Y1
- 10 receptor that has been proposed to regulate feeding behavior. One of the key pharmacological features which distinguish Y1 and Y2 is the fact that the Y1 receptor (and not the Y2 receptor) responds to an analog of NPY modified at residues 31 and 34 ([Leu31,Pro34]NPY), whereas the Y2 receptor (and not the Y1 receptor) has high affinity for the NPY peptide carboxyl-terminal fragment NPY-(13-36)(Fuhlendorff, J.,
- 15 U. Gether, L. Aakerlund, N. Langeland-Johansen, H. Thogersen, S. G. Melberg, U. B. Olsen, O. Thastrup, and T. W. Schwartz. [Leu31,Pro34]Neuropeptide Y: A specific Y1 receptor agonist. *Proc. Natl. Acad. Sci. USA* 87:182-186 (1990)).

Experimental and clinical observations have supported the concept that neuropeptides play central roles in neurotransmission as well as the regulation of

20 secretory functions of adenohypophyseal, pancreatic, adrenalcortical and gut cells. Among the thirty or so neuropeptides that have been implicated in neuronal function in the mammalian central nervous system, several have also been suggested to function as neurotransmitters or neuromodulators primarily in afferent neurons.

An additional action of NPY is to decrease cardiac contractility (inotropy). This is an extremely important action of NPY, because it is known that, under many circumstances in which inotropy is decreased, diseases of life-threatening importance, *e.g.* congestive heart failure and cardiogenic shock, are associated with
5 probable increased release of NPY into the blood. Prevention of NPY release, using a presynaptic NPY agonist, or NPY's action, using a postsynaptic NPY antagonist, may be beneficial in these disease states.

NPY has also been reported to produce coronary artery vasoconstriction and thereby may decrease myocardial blood flow resulting in
10 myocardial ischemia. Such a circumstance can result in angina pectoris or, under more severe circumstances, may result in myocardial infarction and death. In recent years, several classes of drugs have proven effective in dilating coronary arteries to prevent such events. The use of analogs of NPY are expected to prove useful in treatment of such problems.

15 U.S. Pat. No. 4,297,346, Rips, discloses therapeutic agents referred to as 'pseudopeptides' being formed from at least one peptide radical connected by a peptide bond to a therapeutically active molecule or derivative of a therapeutically active molecule. The therapeutic agents of the invention may be in the form of derivatives such as salts, esters and amides. The basis of action of the agents of the
20 invention is the ability of the agents of the invention to cross bodily biological barriers because of the basically peptide structure of the agents. The invention also includes the preparation of the agents of the invention.

U.S. Pat. No. 5,328,899, Boublik *et al.*, issued Jul. 12, 1994, discloses NPY peptide analogs. Human Neuropeptide Y (NPY) has the formula: H-Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro- Ala-Glu-Asp-Met-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile- Thr-Arg-Gln-Arg -Tyr-NH₂. Porcine
5 and rat NPY have the same sequence except for Leu instead of Met in the 17-position. Porcine PYY is homologous having 11 different residues. NPY analogs and N-terminally-shortened fragments, *e.g.* NPY(18-36), which contain one or more specific D-isomer substitutions for the naturally occurring residues (as well as pharmaceutically acceptable salts thereof), dispersed in a pharmaceutically acceptable
10 liquid or solid carrier, can be administered to mammals, including humans, to substantially lower blood pressure over an extended period of time or to counteract hypertension.

U.S. Pat. No. 4,839,343, Waeber *et al.*, issued Jun. 13, 1989, discloses a preparation containing hexatriacontapeptides suitable for intravenous administration
15 to human or other animal subjects which are effective in treating life-threatening hypotension as encountered in bacteremic, anaphylactic or cardiogenic shock.

Several references have disclosed CCK agonists or analogs of CCK-8. For example, U.S. Pat. No. 4,490,364, issued Dec. 25, 1984 to Rivier, discloses heptapeptide, octapeptide and nonapeptide analogs of CCK-8 as CCK agonists for
20 stimulating gallbladder contractions, arresting the secretion of gastric acid and treating convulsions. J. D. Rosamond in European Patent Application EP381,340, published Aug. 8, 1990, and in European Patent Application EP268,297, published May 25,

1988, discloses hepta- and octapeptides with sulfate ester groups which are useful for treating obesity.

U.S. Pat. No. 5,270,302, Shiosaki *et al.*, issued Dec. 14, 1993,

discloses derivatives of tetrapeptides as CCK agonists which are selective and potent

- 5 Type-A CCK receptor agonists useful in the treatment of gastrointestinal disorders (including gallbladder disorders), central nervous system disorders, insulin-related disorders and pain, as well as in appetite regulation.

None of these references individually or collectively teach or suggest the present invention.

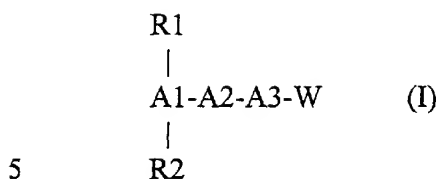
10

Summary of the Invention

- This invention relates generally to dipeptides and tripeptides and to methods for pharmaceutical treatment of mammals using analogs of such dipeptides and tripeptides. More specifically, the invention relates to tripeptides and their
- 15 analogs, to pharmaceutical compositions containing such dipeptides and tripeptides and to methods of treatment of mammals using such dipeptides and tripeptides. In addition, the invention relates to methods of treatment of mammals using such dipeptides and tripeptides for control of appetite, blood pressure, cardiovascular response, libido, and circadian rhythm.

20

The present invention is directed to neuropeptide Y ("NPY") receptor antagonists and agonists. In one aspect, the present invention features compounds having the formula:



wherein:

each R1 and R2, independently, is H, C1-C12 alkyl (*e.g.*, methyl), C6-C18 aryl (*e.g.*, phenyl), C1-C18 acyl (*e.g.*, formyl, acetyl, and myristoyl), C7-C18 aralkyl (*e.g.*, benzyl), C7-C18 alkaryl (*e.g.*, p-methylphenyl) or a dihydrotrigonellinate group;

A1 is a D or L-amino acid selected from the group consisting of Cys, Leu, Dap, Trp, Gln, a tethered amino acid with an indole ring (*e.g.*, N-Me-Trp), Phe, Hyp, any Trp derivative (*e.g.*, 2 chlorotryptophan, or Tcc); C α Me-Trp, C α Me-Gln, Des-amino-Trp, Pyr, Bth, Nal, Tcc, Asn, Nva, Abu, Tyr, Tic-OH, Phe, Tip, and Dip;

A2 is a D or L-amino acid selected from the group consisting of Cys, Trp, Arg, N-Me-Arg, C α Me-Arg, Orn, Cit, hArg(R)₂ [where R is selected from the group consisting of hydrogen, alkyl, aryl, aralkyl, or alkylaryl], Lys- ϵ -NH-R [where R is selected from the group consisting of hydrogen, alkyl, aryl, aralkyl, or alkylaryl];

A3 is a D or L-amino acid selected from the group consisting of Glu, N-Me-Tyr, C α Me-Tyr, Tic-OH, Tic, Dip, Trp, Phe, des-carboxylic-Tyr (tyramine), and Tyr-(R) [where R is hydrogen or a lipophilic group, *e.g.*, myristoyl, cholesteryl, t.Bu, *etc.*];

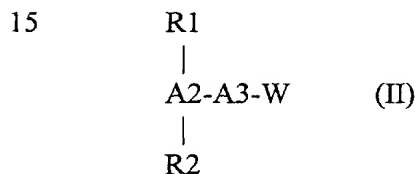
W is -OH, -N-R₃R₄, or OR₅ (where R₃, R₄, and R₅ , independently, is H, C₁-C₁₂ alkyl (*e.g.*, methyl), C₆-C₁₈ aryl (*e.g.*, phenyl), C₁-C₁₂ acyl (*e.g.*, formyl, acetyl, and myristoyl), C₇-C₁₈ aralkyl (*e.g.*, benzyl), or C₇-C₁₈ alkaryl (*e.g.*, p-methylphenyl); or a pharmaceutically acceptable salt thereof; and

5

each bond between two amino acids or amino acid derivatives, represented by a dash (" - "), can be either a peptide bond or a pseudopeptide bond or a pharmaceutically acceptable salt thereof.

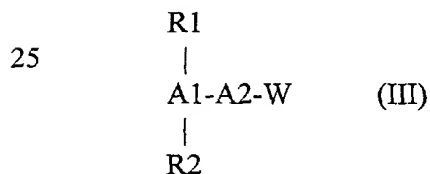
- 10 Preferred compounds of formula (I) include those in which A₁ is Try or a pharmaceutically acceptable salt thereof.

In another aspect, the invention features a compound having the formula:



20

In another aspect, the invention features a compound having the formula:



30

In another aspect, the invention features a compound having the formula:

Cyclo-[-A1-A2-A3-], (IV)

Cyclo-[-A1-A2-A3-A1-A2-A3-], or (V)

Cyclo-[-A1-A2-A3-A3-A2-A1-]. (VI)

In another aspect, the invention features a compound having the
5 formula:

Ac-[A1-A2-A3]_n-NH₂ (VII)

(n = 1, 2, 3) (A Tandem Peptide)

In another aspect, the invention features dimers of compounds having
the formula (I-III). Dimers may be prepared by dimerizing compounds of formula (I-
10 III) with dicarboxylic acids (*e.g.*, succinic acid), cystine, or diaminodicarboxylic acid
(*e.g.*, 2,6-diaminopimelic acid).

In another aspect, the invention features a compound having the
formula of (I-VII) conjugated to carriers.

In another aspect, the invention features a method of controlling the
15 blood pressure of a subject experiencing hypertension.

In another aspect, the invention features a method of controlling a NPY
physiological response in the cardiovascular system, including blood pressure, of a
subject.

In other preferred embodiments, a compound of formula (selected from
20 I thru VII) may have one or more pseudopeptide bonds. The abbreviation psi ("ψ") is
the designation for a pseudopeptide bond (*e.g.*, ("ψ")("CH₂NH") indicates that the
amide ("C(O)NH-") bond of a peptide has been replaced by the reduced form ("CH₂NH-").

In other preferred embodiments, a therapeutically effective amount of a compound of formula (selected from I thru VII) and a pharmaceutically acceptable carrier substance together form a therapeutic composition capable of suppressing an NPY mediated physiological response.

5

Brief Description of the Figures

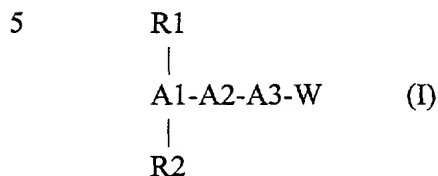
Figure 1. A graph representing the feeding patterns of animals treated with the tripeptides by intrahypothalamic cannulae. The compounds tested include
10 control (artificial cerebral spinal fluid, CSF), T-83 (Ac-Tcc-Arg-Tyr-NH₂), T-84 (N-a-indole-3-acetyl-Arg-Tyr-NH₂), and T-91 (Ac-Trp-Arg-Tic(OH)-NH₂).

Figure 2. A graph representing the feeding patterns of animals treated with the tripeptides by intrahypothalamic cannulae. The compounds tested include
15 control (artificial cerebral spinal fluid, CSF), T-45 (Bis(34/34') {[Cys34]NPY(34-36)}), WX-66 (Ac-D-Trp-Arg-Tyr-NH₂), and WX-126 (Ac-Gln-Arg-Tyr-NH₂).

Figure 3. A graph representing the feeding patterns of animals treated with the tripeptides by intrahypothalamic cannulae. The compounds tested include
20 control (artificial cerebral spinal fluid, CSF) and T-34 (N-Ac-Trp-Arg-Tyr-NH₂).

Detailed Description of the Invention

The present invention is directed to neuropeptide Y ("NPY") receptor antagonists and agonists. In one aspect, the present invention features compounds having the formula:



wherein:

each R1 and R2, independently, is H, C1-C12 alkyl (*e.g.*, methyl), C6-C18 aryl (*e.g.*, phenyl), C1-C18 acyl (*e.g.*, formyl, acetyl, and myristoyl), C7-C18 aralkyl (*e.g.*, benzyl), C7-C18 alkaryl (*e.g.*, p-methylphenyl) or a dihydrotrigonellinate group;

A1 is a D or L-amino acid selected from the group consisting of Cys, Leu, Dap, Trp, Gln, a tethered amino acid with an indole ring (*e.g.*, N-Me-Trp), Phe, Hyp, any Trp derivative (*e.g.*, 2 chlorotryptophan, or Tcc); C α Me-Trp, C α Me-Gln, Des-amino-Trp, Pyr, Bth, Nal, Tcc, Asn, Nva, Abu, Tyr, Tic-OH, Phe, Tip, and Dip;

A2 is a D or L-amino acid selected from the group consisting of Cys, Trp, Arg, N-Me-Arg, C α Me-Arg, Orn, Cit, hArg(R)₂ [where R is selected from the group consisting of hydrogen, alkyl, aryl, aralkyl, or alkylaryl], Lys- ϵ -NH-R [where R

is selected from the group consisting of hydrogen, alkyl, aryl, aralkyl, or alkylaryl];

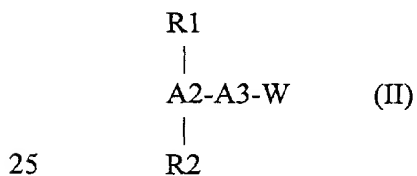
A3 is a D or L-amino acid selected from the group consisting of Glu, N-Me-Tyr, CaMe-Tyr, Tic-OH, Tic, Dip, Trp, Phe, des-carboxylic-Tyr (tyramine), and Tyr-
5 (R) [where R is hydrogen or a lipophilic group, *e.g.*, myristoyl, cholesteryl, t.Bu, *etc.*];

W is -OH, -N-R3R4, or OR5 (where R3, R4, and R5, independently, is H, C1-C12
alkyl (*e.g.*, methyl), C6-C18 aryl (*e.g.*, phenyl), C1-C12 acyl (*e.g.*, formyl,
10 acetyl, and myristoyl), C7-C18 aralkyl (*e.g.*, benzyl), or C7-C18 alkaryl (*e.g.*, p-methylphenyl); or a pharmaceutically acceptable salt thereof; and

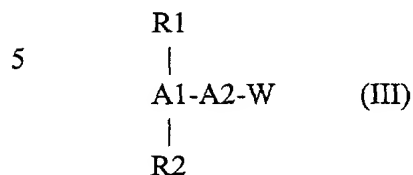
each bond between two amino acids or amino acid derivatives, represented by a
dash ("—"), can be either a peptide bond or a pseudopeptide bond or a
15 pharmaceutically acceptable salt thereof.

Preferred compounds of formula (I) include those in which A1 is Try or a
pharmaceutically acceptable salt thereof.

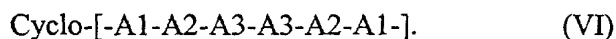
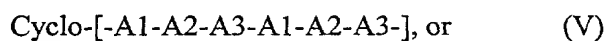
In another aspect, the invention features a compound having the
20 formula:



In another aspect, the invention features a compound having the formula:



10 In another aspect, the invention features a compound having the formula:



15 In another aspect, the invention features a compound having the formula:



(n = 1, 2, 3) (A Tandem Peptide)

20 In another aspect, the invention features dimers of compounds having the formula I-III) prepared by dimerizing the compound with dicarboxylic acids (*e.g.*, succinic acid), cystine, or diaminodicarboxylic acid (*e.g.*, 2,6-diaminopimelic acid).

In another aspect, the invention features a compound having the formula of (I) could be conjugated to carriers, *e.g.*, cationized albumin
25 (Endocrinology 126:977-984 (1990); J. Pharmacol Exp. Therao. 268:791-796 (1994), incorporated herein by reference in their entirety) or polylysine, *e.g.*, MAP.

In another aspect, the invention features a method of controlling the blood pressure of a subject experiencing hypertension which comprises administering to said subject the compound of formula (I-VII).

In another aspect, the invention features a method of controlling a NPY
5 physiological response in the cardiovascular system, including blood pressure, of a subject comprising administering to said subject the compound of formula (I-VII).

In other preferred embodiments, a compound of formula (selected from I thru VII) may have one or more pseudopeptide bonds. The abbreviation psi ("ψ") is the designation for a pseudopeptide bond (*e.g.*, ("ψ")("CH₂NH")) indicates that the
10 amide ("C(O)NH-") bond of a peptide has been replaced by the reduced form ("CH₂NH-") or less preferably that of C(O)NH is replaced with any of CH₂-S, CH₂-O, CH₂-CH₂, CH₂-CO, or CH₂ CH₂). For example, Trp- ("ψ")(CH₂NH)Lys represents a tryptophan residue bonded to a lysine residue wherein the amide bond is reduced. A detailed discussion of the chemistry of pseudopeptide bonds is given in Coy *et al.*
15 (1988) *Tetrahedron* 44: 835-841, incorporated herein in its entirety by reference.

In other preferred embodiments, a therapeutically effective amount of a compound of formula (selected from I thru VII) and a pharmaceutically acceptable carrier substance, *e.g.*, magnesium carbonate or lactose, together form a therapeutic composition capable of suppressing an NPY mediated physiological response. This
20 composition can be in the form a pill, tablet, capsule, liquid, or sustained released tablet for oral administration; or a liquid for nasal administration as drops or spray; or a liquid for intravenous, subcutaneous, parenteral, or intraperitoneal administration.

Another preferred form for administration biodegradable sustained-release composition for intramuscular administration to a subject in need of the composition. Preferably, the composition includes a lipophilic salt and is suitable for administration in the form of an oil emulsion or dispersion to a subject in need of the
5 composition.

In yet another aspect, the invention features methods for controlling an NPY mediated physiological response in a subject; such methods involve administering one or more of the above mentioned compounds to a subject in a dosage effective to control blood pressure, the appetite, the libido, cardiovascular
10 function, and circadian rhythm.

The symbol A1, A2, A3, and the like; and Tyr, Lys or the like, as found in a peptide sequence herein stands for an amino acid residue, *e.g.*, =N-CH(R)-CO- when it is at the N-terminus, or -NH-CH(R)-CO- when it is at any other position, where R denotes the side chain (or identifying group) of an amino acid or its residue.
15 For example, R is CH₂COOH for Asp, R is -H for Gly, R is -CH₂OH for Ser, R is -CH₃ for Ala and R is -CH₂CH₂CH₂CH₂NH₂ for Lys.

As set forth above and for convenience in describing this invention, the conventional and nonconventional abbreviations for the various amino acids are used. They are familiar to those skilled in the art; but for clarity are listed below. All
20 peptide sequences mentioned herein are written according to the usual convention whereby the N terminal amino acid is on the left and the C-terminal amino acid is on the right. A short line between two amino acid residues indicates a peptide bond.
Abbreviations (common):

Asp = D = Aspartic Acid
 Ala = A = Alanine
 Arg = R = Arginine
 Asn = N = Asparagine
 5 Cys = C = Cysteine
 Gly = G = Glycine
 Glu = E = Glutamic Acid
 Gln = Q = Glutamine
 His = H = Histidine
 10 Ile = I = Isoleucine
 Leu = L = Leucine
 Lys = K = Lysine
 Met = M = Methionine
 Phe = F = Phenylalanine
 15 Pro = P = Proline
 Ser = S = Serine
 Thr = T = Threonine
 Trp = W = Tryptophan
 Tyr = Y = Tyrosine
 20 Val = V = Valine

Abbreviations (uncommon):

Aoc = (8-aminooctanoic acid:
 Orn = Ornithine
 25 Nal = 2-naphthylalanine
 Thi = 2-thienylalanine
 Pcp = 4-chlorophenylalanine
 Bth = 3-benzothienylalanine
 Bip = 4,4'-biphenylalanine
 30 Tic = tetrahydroisoquinoline-3-carboxylic acid
 Aib = aminoisobutyric acid
 Anb = alpha-aminonormalbutyric acid
 Dip = 2,2-diphenylalanine
 Ac₆c = 1-aminocyclohexanecarboxylic acid
 35 D-Pal = beta-(3-pyridyl)alanine;
 Tcc = tetrahydrocarbolene-carboxylic acid
 Abu = α-aminonormalbutyric acid
 hArg(Pr)₂ = N,N'-guanidino-(dipropyl)-homoarginine
 Tic-OH = 1,2,3,4 tetrahydroisoquinoline-7-hydroxy-3-carboxylic acid
 40 Dip = 3,3-diphenylalanine
 2-Nal = 3(2-naphthylalanine)
 Tfp = Paratrimethyl phenylalanine
 Fla = 3-(9-Fluorenyl)alanine
 Flg = 9-Fluorenylglycine
 45 Cit = Citruline
 Adp = 2,5-diaminoadipic acid

Pim = 2,6-diaminopimelic acid
Sub = 2,7-diaminosuberic acid

The compounds of the invention are useful in treating any number of
5 illnesses that involve eating disorders, cardiovascular function, alterations in sexual
function, as well as disorders of sleep and circadian rhythms (see, *e.g.*, *Harrison 's*
Principles of Internal Medicine, McGraw-Hill Inc., New York, 12th ed.).

Other features and advantages of the invention will be apparent from
the following description of the preferred embodiments thereof, and from the claims.

10 The present invention is also directed to pharmaceutical compositions
comprising a therapeutically-effective amount of a compound of formula (I-VII) and a
pharmaceutically-acceptable carrier or diluent, as well as to a method of treating
gastrointestinal disorders (including gallbladder disorders), central nervous system
disorders, insulin-related disorders and pain, or of regulating appetite in humans and
15 lower mammals, by administration of a compound of formula (I-VII).

The terms "C2-C4-alkenyl" and "C2-C6-alkenyl" as used herein refer
to a 2 to 4 to 6 straight- or branched-chain of carbon atoms which contains a carbon-
carbon double bond, such as allyl, propenyl, butanol, isoprenyl and the like.

The terms "C1-C18-alkyl" as used herein refer to straight or branched
20 chain alkyl radicals having from 1 to 18 carbon atoms including, but not limited to,
methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, pentyl, neopentyl hexyl,
and the like.

The term "C6-C18-aryl" as used herein refers to phenyl or to a
"bicyclic carbocyclic" group or "bicyclic carbocycle" having two fused carbocyclic
25 rings, each ring having 5, 6 or 7 carbon atoms, and each ring being fully saturated,

partially saturated or aromatic. Bicyclic carbocyclic groups include, but are not limited to, naphthyl, tetrahydronaphthyl, decalin, indanyl, indenyl and the like.

The term "C7-C18-arylalkyl" as used herein refers to an aryl group appended to a C1-C4-alkyl radical including, but not limited to, benzyl, phenethyl, 5 naphthylmethyl and the like.

The term "bicyclic heterocycle" as used herein refers to a group having two fused rings, one or both of which are heterocyclic rings as defined herein. When both rings are not heterocyclic, the other ring is carbocyclic and is saturated, partially saturated or aromatic, preferably a benzene ring. Bicyclic heterocyclic groups can be 10 unsubstituted or monosubstituted or disubstituted with substituents independently selected from hydroxy, halo, oxo (=O), amino, C1-C4-alkylamino, di-(C1-C4)-alkylamino, C1-C4-alkoxy, thio-C1-C4-alkoxy, carboxy, C1-C4-alkoxycarbonyl, C1-C4-alkyl, C3-C8-cycloalkyl, -OSO₃H and halo-C1-C4-alkyl. Examples of bicyclic heterocycles include indole, 5-hydroxyindole, quinoline, isoquinoline, 15 tetrahydroisoquinoline, quinoxaline, benzimidazole, benzofuran, and the like.

The term "cyclo-C3-C10-alkyl" as used herein refers to an aliphatic monocyclic of 3 to 10 or bicyclic group having 6 to 10 carbon atoms including, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclooctyl, adamantyl, and the like.

20 The term "halo" or "halogen" as used herein refers to chloro, bromo, iodo or fluoro.

The term "halo-C1-C4-alkyl" as used herein refers to a lower alkyl radical in which one to three hydrogen atoms have been replaced by a halogen including, but not limited to, chloromethyl, 2-fluoroethyl, trifluoromethyl and the like.

The term "monocyclic heterocyclic group" or "monocyclic heterocycle" as used herein refers to any 3- or 4-membered ring containing a heteroatom selected from oxygen, nitrogen and sulfur, or a 5- or 6-membered ring containing carbon atoms and one, two or three nitrogen atoms; one nitrogen and one sulfur atom; or one nitrogen and one oxygen atom; wherein the 5-membered ring has 0-2 double bonds and the 6-membered ring has 0-3 double bonds; wherein the nitrogen and sulfur heteroatoms may optionally be oxidized; and wherein the nitrogen heteroatom may optionally be quaternized. Heterocycles include, but are not limited to, pyridyl, imidazolyl, furyl, thienyl, pyrazinyl, pyrrolyl, pyrimidyl and the like. Heterocyclics may be unsubstituted or mono- or disubstituted with substituents independently selected from hydroxy, halo, oxo (=O), amino, C1-C4-alkylamino, (C1-C4)2-alkylamino, C1-C4-alkoxy, thio-C1-C4-alkoxy, carboxy, C1-C4-alkoxycarbonyl, C1-C4-alkyl, C3-C8-cycloalkyl, -OSO₃H and halo-C1-C4-alkyl.

The compounds of formula (I) contain two or more asymmetric carbon atoms and thus can exist as pure diastereomers, mixtures of diastereomers, diastereomeric racemates or mixtures of diastereomeric racemates. The present invention includes within its scope all of the isomeric forms. In keeping with standard peptide nomenclature, J. Biol. Chem., 1969, 243:3557-59, abbreviations for amino acid residues are used herein.

It is noted that all amino acid residue sequences are represented herein by formulae whose left to right orientation is in the conventional direction of amino-terminus to carboxy-terminus.

5 Administration

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated, the particular treatment and the particular mode of administration.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy.

However, generally speaking the following guidelines will suffice.

When a compound of formula (I) is used as an agonist of NPY in a human subject, the total daily dose administered in single or divided doses may be in amounts, for example, from 0.001 to 1000 mg a day and more usually 1 to 1000 mg. Dosage unit compositions may contain such amounts of submultiples thereof to make up the daily dose.

The compounds useful in the present inventive method may be administered by any suitable means. One skilled in the art will appreciate that many suitable methods of administering the compounds to an animal in the context of the present invention, in particular a human, are available, and, although more than one

route may be used to administer a particular compounds, a particular route of administration may provide a more immediate and more effective reaction than another route.

The composition according to the present invention may be formulated
5 for administration by any suitable route such as the oral, rectal, nasal, topical (dermal) or parenteral administration route. Thus, the composition may be in the form of tablets, capsules, suspensions, emulsions, solutions, injectables, suppositories, sprays, aerosols and in other suitable form.

Formulations for oral use include tablets which contain the active
10 ingredient in admixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents, such as calcium carbonate, sodium chloride, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, potato starch or alginic acid; binding agents, for example, starch, gelatin or acacia; and lubricating agents, for example, magnesium
15 stearate, stearic acid or talc. Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants *etc.* The tablets may be uncoated or they may be coated by known techniques, optionally to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or
20 glyceryl distearate may be employed.

Formulations for oral use may also be presented as chewing tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft

gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil.

Powders, dispersible powders or granules suitable for preparation of an aqueous suspension by addition of water are also convenient dosage forms of the present invention. Formulation as a suspension provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents are, for example, naturally-occurring phosphatides, as *e.g.* lecithin, or condensation products of ethylene oxide with *e.g.* a fatty acid, a long chain aliphatic alcohol or a partial ester derived from fatty acids and a hexitol or a hexitol anhydrides, for example, polyoxyethylene stearate, polyoxyethylene sorbitol monooleate, polyoxyethylene sorbitan monooleate *etc.* Suitable suspending agents are, for example, sodium carboxymethylcellulose, methylcellulose, sodium alginate *etc.*

The present agents can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to the tetrapeptide of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic.

Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 et seq.

The pharmaceutical formulation may also be administered parenterally
5 (intravenous, intramuscular, subcutaneous or the like) in dosage forms or formulations containing conventional, non-toxic pharmaceutically acceptable carriers and adjuvants. The formulation and preparation of such compositions is well-known to those skilled in the art of pharmaceutical formulation.

For parenteral use, the pharmaceutical compositions according to the
10 invention may comprise the thermogenic compounds in the form of a sterile injection. To prepare such a composition, the compounds are dissolved or suspended in a parenterally acceptable liquid vehicle. Among acceptable vehicles and solvents that may be employed are water, water adjusted to a suitable pH by addition of an appropriate amount of hydrochloric acid, sodium hydroxide or a suitable buffer, 1,3-
15 butanediol, Ringer's solution and isotonic sodium chloride solution. The aqueous formulation may also contain one or more preservatives, for example, methyl, ethyl or n-propyl p-hydroxybenzoate.

For the rectal application, suitable dosage forms for a composition according to the present invention include suppositories (emulsion or suspension
20 type), and rectal gelatin capsules (solutions or suspensions). In a typical suppository formulation, the compounds are combined with an appropriate pharmaceutically acceptable suppository base such as cocoa butter, esterified fatty acids, glycerinated gelatin, and various water-soluble or dispersible bases like polyethylene glycols and

polyoxyethylene sorbitan fatty acid esters. Various additives like *e.g.* enhancers or surfactants may be incorporated.

For the nasal application typical dosage forms for a composition according to the present invention include nasal sprays and aerosols for inhalation. In
5 a typically nasal formulation, the active ingredients are dissolved or dispersed in a suitable vehicle. The pharmaceutically acceptable vehicles and excipients and optionally other pharmaceutically acceptable materials present in the composition such as diluents, enhances, flavoring agents, preservatives, *etc.*, are all selected in accordance with conventional pharmaceutical practice in a manner understood by the
10 persons skilled in the art of formulating pharmaceuticals.

The pharmaceutical compositions according to the invention may also be administered topically on the skin for percutaneous absorption in dosage forms or formulations containing conventionally non-toxic pharmaceutically acceptable carriers and excipients including microspheres and liposomes. The formulations
15 include creams, ointments, lotions, liniments, gels, hydrogels, solutions, suspensions, pastes, plasters and other kinds of transdermal drug delivery systems. The pharmaceutically acceptable carriers or excipients may include emulsifying agents, antioxidants, buffering agents, preservatives, humectants, penetration enhancers, chelating agents, gelforming agents, ointment bases, perfumes and skin protective
20 agents.

Examples of emulsifying agents are naturally occurring gums, *e.g.*, gum acacia or gum tragacanth, naturally occurring phosphatides, *e.g.*, soybean lecithin and sorbitan monooleate derivatives.

Examples of antioxidants are butylated hydroxy anisole (BHA), ascorbic acid and derivatives thereof, tocopherol and derivatives thereof and cysteine.

Examples of preservatives are parabens and benzalkonium chloride.

Examples of humectants are glycerin, propylene glycol, sorbitol and
5 urea.

Examples of penetration enhancers are propylene glycol, DMSO, triethanolamine, N,N-dimethylacetamide, N,N-dimethylformamide, 2-pyrrolidone and derivatives thereof, tetrahydrofurfuryl alcohol and AZONE®.

Examples of chelating agents are sodium EDTA, citric acid and
10 phosphoric acid.

Examples of gel forming agents are Carbopol, cellulose derivatives, bentonit, alginates, gelatin and PVP.

Examples of ointment bases are beeswax, paraffin, cetyl palmitate, vegetable oil, sorbitan esters of fatty acids (Span), polyethyleneglycols, and
15 condensation products between sorbitan esters of fatty acids and ethylene oxide, *e.g.*, polyoxyethylene sorbitan monooleate (Tween).

The formulation and preparation of the above-mentioned compositions is well-known to those skilled in the art of pharmaceutical formulation. Specific formulation can be found in "Remington's Pharmaceutical Sciences" incorporated
20 herein by reference.

In one aspect the present invention relates to a method for treatment of overweight or obesity in individuals, in particular in humans or for reducing the

adipose tissue mass/lean mass body mass ratio of an individual, in particular a human or a domestic animal.

In the present context the term "overweight" is used as an indication of a body with a weight exceeding the "desirable weight", whereas the term "obesity" is used when the body weight is 20% or more above the "desirable weight". Desirable weights for humans are given by the Council on Scientific Affairs (1) defining the desirable weights for humans according to Metropolitan Height and Weight Tables as the midpoint of the range of the medium-frame individuals.

In another aspect, the present invention relates to a method for the treatment of diseases which are complications to overweight or obesity. These diseases or conditions include diabetes mellitus type II, hypercholesterolemia, hypertriglyceridaemia and hypertension.

In another aspect, the present invention also relates to a method of reducing adipose tissue mass/lean body mass ratio or treating overweight or obesity or complications thereof by means of subjecting the individuals to a diet regimen. The diet regimen into which the individuals may be subjected in connection with the administration of the composition may include a low carbohydrate, a low fat and a low energy regimen, *e.g.*, a diet of from 800-2500 kcal/day.

20 Veterinary Use

The compositions according to the invention can also be administered to domestic animals in order to improve the performance of the animal (daily weight gain and feed utilization) or to improve carcass quality or both. Carcass quality is

generally improved when the fat tissue mass/lean mass body mass ratio is decreased, *i.e.*, when the body content of meat is increased *e.g.*, at the expense of the body content of fat.

- The improvements in performance and carcass quality are suggested to
- 5 be caused by a reduced fat accretion and/or by an increased skeletal muscle accretion.
- In growing animals, the amount of lipid present is suggested to be governed by the relative rates of lipolysis and lipogenesis. Stimulation of lipolysis and/or inhibition of lipogenesis in fat tissue may lead to a reduced fat accretion. In vivo and in vitro
- 10 lipolysis and inhibit lipogenesis in fat tissue leading to a reduced fat accretion.

- Administration to an animal of the compositions according to the present invention may be useful in order to increase the lean body mass at the expense of body fat, particularly in domestic animals like pigs, hogs, cattle, sheep and poultry. The composition may be given in admixture with the feed in a suitable dose
- 15 corresponding to the size of the animal.

Peptide Synthesis

- The peptides can be synthesized by any suitable method, such as by exclusively solid-phase techniques, by partial solid-phase techniques, by fragment
- 20 condensation or by classical solution addition. A detailed description of these methods is contained in "The Peptides, Vol. 1", Gross and Meinenhofer, Eds., Academic Press, New York, 1979. Coupling methods employed include the carbodiimide method (1,3-dicyclohexylcarbodiimide [DCC], 1-(3-

dimethylaminopropyl-3-ethylcarbodiimide hydrochloride [EDCI]) with the option of racemization preventing additives (1-hydroxybenzotriazole [HOBT]), the mixed anhydride method, the azide method, the acid chloride method, the symmetrical anhydride method, the use of bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl),
5 and the active ester method (N-hydroxysuccinimide esters, 4-nitrophenol esters, 2,4,5-trichlorophenol esters, and the like).

For example, the techniques of exclusively solid-phase synthesis are set forth in the textbook "Solid-Phase Peptide Synthesis" Stewart & Young, Freeman & Co., San Francisco, 1969, and are exemplified by the disclosure of U.S. Pat. No.
10 4,105,603, issued Aug. 8, 1978 to Vale *et al.* The fragment condensation method of synthesis is exemplified in U.S. Pat. No. 3,972,859 (Aug. 3, 1976). Other available syntheses are exemplified by U.S. Pat. No. 3,842,067 (Oct. 15, 1974) and U.S. Pat. No. 3,862,925 (Jan. 28, 1975). All of the above references are incorporated herein by reference.

15 Synthesis by the use of recombinant DNA techniques, for purposes of this application, should be understood to include the suitable employment of a structural gene coding for all of the tripeptide to transform a microorganism, using an expression vector including a promoter and operator together with such structural gene, and causing such transformed microorganism to express the peptide or such a
20 synthetic peptide fragment. A non-human animal may also be used to produce the peptide by gene-farming using such a structural gene in the microinjection of embryos as described in U.S. Pat. No. 4,870,009 issued Sep. 26, 1989, incorporated herein by reference.

When the peptides are not prepared using recombinant DNA technology, they are preferably prepared using solid phase synthesis, such as that described by Merrifield, J. Am. Chem. Soc., 85, p 2149 (1964), although other equivalent chemical syntheses known in the art can also be used as previously mentioned. Solid-phase synthesis is commenced from the C-terminus of the peptide by coupling a protected alpha-amino acid to a suitable resin as generally set forth in U.S. Pat. No. 4,244,946 issued Jan. 21, 1981 to Rivier *et al.*, the disclosure of which is incorporated herein by reference. Such a starting material for NPY analogs can, for example, be prepared by attaching alpha-amino- and side-chain-protected Tyr to a BHA resin.

The compounds of the invention may be prepared by stepwise coupling of the amino acids or by coupling together fragments of dipeptide length or greater. Thus, the free carboxylic acid moiety from one amino acid or peptide fragment is activated and allowed to condense with the free nitrogen group of the second amino acid or peptide fragment. The coupling reactions are conducted in solvents such as methylene chloride (CH_2Cl_2), tetrahydrofuran (THF), dimethylformamide (DMF) or other such solvents.

During the coupling process, the non-participating carboxylic acids or amines on the reacting set of amino acids or peptide fragments are protected by a protecting group which can be selectively removed at a later time if desired. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, incorporated herein in its entirety by reference. Thus, useful protective groups

for the amino group are benzyloxycarbonyl (Cbz), t-butyloxycarbonyl (t-BOC), 2,2,2-trichloroethoxycarbonyl (Troc), t-amylloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-(trichlorosilyl)ethoxycarbonyl, 9-fluorenylmethoxycarbonyl (Fmoc), phthaloyl, acetyl (Ac), formyl, trifluoroacetyl, and the like.

5 Examples of useful protective groups for the carboxylic acid include esters, such as methyl, ethyl, benzyl, t-butyl, 2,2,2-trichloroethyl, allyl, 4-nitrobenzyl, and the like. Removal of these protecting groups may be accomplished selectively by employing various acid or base catalyzed hydrolytic, hydrogenolytic, thermal or dissolving metal conditions.

10 Generally, peptides will be synthesized by stepwise solid phase methodology developed by using an automated Applied Biosystem Model 430A peptide synthesizer. Tertiary butyloxy-carbonyl (Boc) amino acids with benzyl or halobenzyl based side chain protecting groups (Asp & Glu with OBzl; Ser & Thr with Bzl; Cys with pMeBzl; Tyr with 2BrZ; Lys with 2ClZ; Arg with Tos; His with Bom; 15 Trp with CHO) will be used in conjunction with phenylacetamidomethyl (PAM) resin. In the case of the synthesis of peptide amides, benzyldrylamine (BHA) or paramethylbenzyldrylamine (MBHA) will be used instead of PAM resin.

Boc-aminoacid-PAM-resin, using Boc-aminoacyloxy-methyl-phenylacetic acid and aminomethyl resin, is available commercially. The Boc- 20 aminoacid-PAM-resin thus prepared eliminates the possibility of chain termination by tri-fluoroacetylation. Attachment to BHA or MBHA resin will be performed by way of preformed symmetrical anhydride.

Coupling and deprotection functions are generally carried out automatically by the instrument. The standard program provided by the manufacturers are modified to incorporate a double coupling procedure, first in DMF and then in CH_2Cl_2 . Altering the polarity of the solvents improves the coupling. All amino acids, except Asn, Gln and Arg, will generally be coupled as preformed symmetrical anhydrides. Asn, Gln and Arg are double coupled as preformed 1-hydroxy-benzotriazole esters to avoid side reactions. Resin samples taken during these reactions may be assayed by quantitative procedure to determine the degree of coupling. Other standard cleaving reagents and conditions for removal of specific alpha-amino protecting groups may be used as described in Schroder & Lubke, "The Peptides" Vol. 1, pp 72-75 (Academic Press 1965), incorporated herein in its entirety by reference.

In the case of coupling unusual amino acids, suitable conditions (solubility, coupling times) will be first developed before using in automated mode. In some cases these couplings will be carried out manually (eg: pseudopeptides, N-Me-amino acids). Pseudopeptide bonds will be incorporated by the methods described earlier. The t-Boc-amino acid aldehyde will be obtained by reducing N-methoxy-N-methylamide derivatives of Boc-amino acids with LiAlH_4 . The aldehyde obtained will be reacted immediately with the α -amino group of the peptide attached to the resin in DMF containing 1.0% HOAC in the presence of an equivalent amount of NaBH_3CN . At the end of the reaction, the presence of secondary amine is tested for with ninhydrin (wine-red color). The secondary amine formed will then be blocked by reacting with 2 equivalents of Z(2-Cl)OSU , 2 equivalents of HOBt, and 4

equivalents of diisopropylethyamine until ninhydrin gives a yellow color. This way the formation of branched peptide is prevented. Coupling of sterically hindered amino acids (eg: N-Me-amino acids, C α MeLeu, Aib) will be effected by a HOAT or HATU which has been shown to be superior to BOP/HOBT.

- 5 For the final cleavage, the N- α -Boc group and the Nin-CHO will be first removed with 50% TFA/CH₂Cl₂ and 20% piperidine-DMF from the protected peptide resin before detaching the target peptide using HF containing p. cresol (5%). If Cys and Met are present, p. thicresol (2.5%) will also be added to the HF reaction mixture. If problems are encountered during the standard HF method, then the
- 10 “low/high” HF procedure will be used.

- The materials are then purified. After initial fractionation on Sephadex G-25, the peptide material will be subjected to reversed phase high performance liquid chromatography (RPLC) on C₁₈ Vydac columns. However, peptides may be first subjected to ion exchange chromatography before RPLC, depending upon the
- 15 heterogeneity of the crude peptide. The homogeneity of the purified product may be confirmed by analytical RPLC using two different solvent systems, amino acid analysis, complete sequencing, and mass spectral analysis.

- For analysis, the peptide resins are hydrolyzed using 12N HCl/HOAc/phenol (2:1:1) for 24 hours at 110°C. The free peptides are hydrolyzed
- 20 for 24 hours in 6N HCl containing 0.1% phenol or 4N methane sulfonic acid at 110°C and are quantified on a Waters Pico Tag system. Peptide hormones and fragments are then subjected to complete sequencing on an automated gas phase sequencer (Applied Biosystem, Model 470A).

For the production of a compound of the invention where any one or several of the constituent amino acids bear an N-alkyl group, specifically methyl, the corresponding N-alkyl amino acid can be prepared via the method described by Benoiton (*Can. J. Chem.*, 1977, 55:906) or Shuman ("Peptides: Proceedings of the 7th American Peptide Symposium", D. Rich, E. Gross, Eds., Pierce Chemical Co., Rockford, Ill. 1981, p 617), wherein the t-BOC- or Cbz-protected amino acid is treated with a base in the presence of a chelating agent such as a crown ether and then quenched with methyl iodide. An alternative method described by Freidinger (*J. Org. Chem.*, 1983, 48:77), in which triethylsilane reduction of the oxazolidinone of an amino acid directly produces the N-methyl derivative may also be utilized.

The reduced carbonyl amide bond surrogates can be prepared in a manner similar to that described by Martinez (*J. Med. Chem.* 1987, 30:1366). The N-alpha -t-BOC protected amino acid (with appropriate protection of side chain functional groups) is converted to the 3,5-dimethylpyrazolide, which is then reduced with lithium aluminum hydride. The resulting aldehyde is then allowed to condense with an amino acid or peptide bearing a free amino terminus. Reduction of the Schiff base which is formed as a result of the condensation is accomplished using sodium cyanoborohydride to yield the desired compound having a reduced amide bond.

Functionalization of the epsilon -amino group of the lysine (Lys) or homologous (*e.g.*, Orn) residue is achieved via activation of the acid fragment as the active ester (N-hydroxysuccinimide, 2,4,5-trichlorophenol, *etc.*) or, if no other free carboxylic acid function is present on the peptide, coupling using any of the methods mentioned above is applicable. In addition, the functionalization of the epsilon -

amino group may be accomplished by reaction with various alkyl and aryl isocyanates, as well as alkyl and aryl isothiocyanates.

The sulfuric acid esterification of the phenolic residues may be conducted using a variety of known reagents such as the pyridine-sulfuric anhydride or the pyridine-sulfur trioxide complex. Use of pyridinium acetyl sulfate as described 5 by Penke and Rivier ("Proceedings of the 8th American Peptide Symposium", V. Hruby, D. Rich, Eds., Pierce Chemical Company, Rockford, IL; 1983; p. 119), may also be applied to prepare the sulfuric acid ester derivative of the tetrapeptides.

The compounds of the present invention can be used in the form of salts derived from inorganic or organic acids. These salts include but are not limited 10 to the following: acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, cyclopentanepropionate, dodecylsulfate, ethanesulfonate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxy-ethanesulfonate, lactate, maleate, 15 methanesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate. Also, the basic nitrogen-containing groups can be quaternized with such agents as loweralkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides, and iodides; dialkyl sulfates like dimethyl, 20 diethyl, dibutyl, and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl

bromides, and others. Water or oil-soluble or dispersible products are thereby obtained.

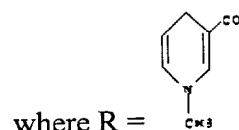
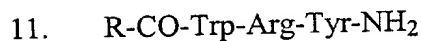
The pharmaceutically acceptable salts of the present invention can be synthesized which contain a basic or acidic moiety by conventional chemical methods. Generally, the salts are prepared by reacting the free base or acid with stoichiometric amounts or with an excess of the desired salt forming inorganic or organic acid or base in a suitable solvent or various combinations of solvents.

Examples of preferred salts are those with therapeutically acceptable organic acids, *e.g.*, acetic, lactic, maleic, citirc, malic, ascorbic, succinic, benzoic, or pamoic acid, as wells as polymeric acids and salts with inorganic acids such as the hydrohalic acids, *e.g.*, hydrocholoric and sulfuric acids.

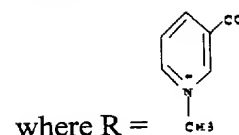
In addition, pseudopeptide bonds may, if desired, may be introduced at various positions, *e.g.*, between amino acid residues A1-A2 or between residues A2-A3. Optically pure Boc-AA-CHO can be obtained in good yields and coupled directly to the -NH₂ group of the peptide resin by published methods (Sasaki *et al.*, *Peptides* 8:119-121, 1987; Fehrentz *et al.*, *Synthesis* pp.676-678, 1983). The secondary amine in the pseudopeptide bond is capped with Z(2-Cl). This is introduced by reacting the peptide resin with Z(2-Cl)-OSU (2 equiv.), HOBT (2 equiv.) and DIEA (4 equiv.) for 10-60 min. The red wine color of ninhydrin with secondary amine turns yellow at the end of capping. Exemplary compounds of the present invention include:

1. Ac-Phe-Arg-Trp-NH₂
2. Ac-Trp-Arg-Phe-NH₂
- 25 3. Ac-Trp-Arg-Tyr-NH₂
4. Ac-Trp-Ctr-Tyr-NH₂
5. Ac-Trp-(Ψ)-Arg-Tyr-NH₂

6. Ac-Trp-Arg-Tyr-(Ψ)-NH₂
7. Ac-Arg-Tyr- NH₂
8. Ac-Trp-Arg-Tyr-[O(PO(OH)₂)]-NH₂
9. (HO)₂PO-CH₂-CO-Trp-Arg-Tyr-NH₂
- 5 10. Ac-Arg-His-Tyr-NH₂



12. R-CO-Trp-Arg-Tyr-NH₂
13. Ac-Trp-Arg-D-Tyr-NH₂
14. Ac-Trp-D-Arg-Tyr-NH₂
- 10 15. Ac-D-Trp-Arg-Tyr-NH₂
16. Ac-D-Trp-D-Arg-D-Tyr-NH₂
17. Trp-Arg-Tyr-NH₂
18. Ac-Trp-Arg-Tyr-OH
19. Ac-Trp-Arg-Tyr-NH-Me
- 15 20. Ac-Trp-Arg-Tyr-NH₂
21. Ac-Arg-Tyr-NH₂
22. Bis(34/34') {[Cys34]NPY(34-36)}
23. Ac-Gln-Arg-Tyr-NH₂
24. H-Gln-Arg-Tyr-NH₂
- 20 25. Ac-Trp-h.Arg((Pr)₂-Tyr-NH₂
26. [Des-α-Amino-Trp]-Arg-Tyr-NH₂
27. Ac-Trp-Arg-Tic(OH)-NH₂
28. N-a-Indole-3-acetyl-Arg-Tyr-NH₂
29. Ac-Tcc-Arg-Tyr-NH₂
- 25 30. Ac-Pro-Arg-Tyr-NH₂
31. Ac-Nal-Arg-Tyr-NH₂
32. Ac-Bth-Arg-Tyr-NH₂
33. Ac-Trp-Arg-Tyr-NH-CH₃
34. Ac-D-Trp-Arg-Tyr-NH₂
- 30 35. Ac-Trp-D-Arg-Tyr-NH₂
36. Ac-Trp-Arg-D-Tyr-NH₂
37. Ac-D-Trp-D-Arg-D-Tyr-NH₂
38. Ac-[-Trp-Arg-Tyr-]₂-NH₂
39. [Ac-Cys-Trp-Arg-Tyr-NH₂]₂
- 35 40. -[-CH₂CO-Trp-Arg-Tyr-NH₂]₂
41. Dap-Ile-Trp-Arg-Glu-Arg-Tyr-NH₂
42. Leu-Ile-Trp-Arg-Glu-Arg-Tyr-NH₂
43. Cyclo[-Trp-Arg-Nva-Arg-Tyr-]
44. RCO-Trp-Arg-Tyr-NH₂
- 40 45. Ac-Trp[CH₂NH]Arg-Tyr-NH₂
46. Ac-Trp-Arg-[CH₂NH]Tyr-NH₂



47. Ac-Arg-Tyr-NH₂
48. Ac-Trp-Arg-NH₂
49. Ac-Tcc-Arg-Tic(OH)-NH₂
50. Cyclo[-Trp-Arg-Tyr-Trp-Arg-Tyr-]
- 5 51. N- α -Ac-Cyclo(29/34)[D-Cys 29,34,Trp 32]NPY(29-36)-NH₂
52. Cyclo(30/34)[Leu30,Trp32,Glu34,]NPY(30-36)-NH₂
53. Cyclo(30/34)[Dap30,Trp32,Glu34,]NPY(30-36)-NH₂

Other analogs of the invention can be prepared as above and tested for
 10 their biological activity effectiveness as antagonists or agonists using the methods
 described below and those commonly known in the art.

FUNCTIONAL ASSAYS

Animals. Cell Lines and Cultures. and Reagents

15 Any suitable in vivo or in vitro system may be utilized to assay and
 test the effectiveness of the compounds of the invention. Such assays may employ in
 vivo methods for evaluating physiological responses, *e.g.*, blood pressure,
 renovascular function, feeding behavior, or circadian rhythm, or in vivo biochemical
 systems evaluating receptor binding in a suitable cell line, *e.g.*, SK-N-MC (ATCC
 20 HBT 10) or SK-N-BE(2) (Barnes *et al. In Vitro*, 17: 619 631, 1981); or in isolated
 cells, *e.g.*, cells isolated from the spleen, kidney, heart or brain. A number of *in vivo*
 and *in vitro* biochemical systems known to those skilled in the art are available for
 testing antagonists to hypothalamic NPY receptors, *e.g.* the Y-1, Y-2, and Y-3 receptor
 categories. Described below are assay methods which can be utilized with cell lines
 25 such as SK-N-MC and SK-N-BE2 or isolated cardiac membranes which possess the
 high-affinity hypothalamic NPY receptor sites. Other systems are also known for
 evaluating NPY antagonists to the hypothalamic NPY receptor, *e.g.* VSM cells

(Sheikh *et al.*, *Am. J. Physiol.* 260: G250-G257, 1991) and HEL cells (Motulsky *et al.* *Amer. J. Physiol.* 255: E880-E885, 1988); Y-2 receptor, *e.g.*, kidney (Sheikh *et al.*, *Am. J. Physiol.* 26:F978-F984), spleen (Lunberg *et al.*, *Eur. J. Pharmacol.* 145:21-29, 1988), dorsal root ganglion (Bleakman *et al.*, *Br. J. Pharmacol.* 103:1781-1789, 1991)
5 and hippocampal cells (Sheikh *et al.*, *J. Biol. Chem.* 265:8304-8310, 1990); and Y-3 receptors, *e.g.*, in cardiac ventricular membranes (Balasubramaniam *et al.*, *Peptides* 11: 545-550, 1990), chromaffin cells, rat gastric mucosa (Michel, M.C., *Trends in Pharmacol. Sci.* 12: 389-394, 1991) and brain stem.

10 In Vitro Biochemical Assays

The ability of the compounds of the invention to act as antagonists of NPY can be demonstrated by any number of methods known in the art. For example, the compounds can be shown to compete with iodinated neuropeptide Y for receptors using the methods described by Lundberg *et al.* (*Eur. J. Pharmacol.* 145: 21-29, 1988);
15 Gordon *et al.* (*J. Neurochemistry* 55:506-513, 1990); Walker *et al.* (*Mol. Pharmacol.* 34:779-792, 1988); Balasubramaniam *et al.* (*Peptides* 10:1283-1286, 1989).

In one example demonstrating antagonists to hypothalamic NPY receptors, rat hypothalamus was isolated and the membranes were prepared for binding and adenylate cyclase studies according to standard methods (Unden *et al.* 1984. *Eur.*
20 *J. Biochem* 145: 525-530; Westlind-Danielsson *et al.*, *Neurosci. Lett.* 74: 237-242 (1987)). Displacement studies are performed in a total volume of 0.25 ml 20 mM HEPES buffer, pH 7.4, containing 1% bovine serum albumin, 0.1% bacitracin, 300 μ M PMSF and 5 KIU/ml aprotinin. In a standard assay, 100 μ g of membrane/tube is

incubated in a shaking water bath at 24 °C for 45 min with [¹²⁵I-Tyr]-NPY (20,000 CPM) as described by Balasubramaniam *et al.*, (*Peptides* 11: 545-550, 1990), in the presence of increasing concentrations of NPY (10 μOsM). At the end of incubation, 1.0 ml of iced cold buffer is added, centrifuged at 10,000 x g for 10 min, and the supernatant removed by aspiration. The tube containing the pellet is counted for bound radioactivity in a micromedic gamma counter.

An example of assaying adenylate cyclase activity of hypothalamic and cerebral cortex membranes is now described.

Adenylate cyclase activity of the hypothalamic and cerebral cortex membranes is determined by incubating 50 μg of membranes in a total volume of 0.20 ml Tris-HCl 30 mM pH 7.4 buffer containing 150 mM NaCl, 8.25 mM MgCl₂, 0.75 mM EGTA, 1.5 theophylline, 20 μg/ml aprotinin, 100 μg/ml bacitracin, 1 mg/ml bovine serum albumin, 1 mM ATP, 20 mM creatine phosphate, 1 mg/ml phosphocreatine kinase, 10 μM isoprenaline, 10 μM GTP, and various concentrations of peptides (0-10 μM). After incubating the mixture at 35 °C for 15 min in a shaking water bath, the reaction is arrested by the addition of 100 μM EDTA and boiling for 3 min. cAMP is extracted and quantitated by radioimmunoassay. All the points in the binding and adenylate cyclase are the means of at least three parallel experiments performed in duplicate.

20

In Vivo Assays

Any suitable *in vivo* model system can be used to evaluate the antagonistic properties of the compounds of the invention. Such models, without

limitation, include those used to evaluate feeding and memory behavior (Flood *et al.*, *Peptides* 10:963-966), and vasoconstriction and hypertension (Balasubramaniam *et al.* *Biochemm et Biophys Acta* 997: 176-188, (1989)).

Thus, in one example, feeding studies are performed using Spraque
5 Dawley rats (350-450 g) with paraventricular hypothalamic cannulae to investigate
effecta of NPY analogs (Chance *et al.*, *Peptides* 10: 1283, 1286 (1989)).

The following Examples set forth preferred methods for synthesizing
tripeptides by the solid-phase technique and generally is in accordance with the
procedure set forth in U.S. Pat. No. 4,415,558 to Vale, *et al.*, issued Nov. 15, 1983,
10 the disclosure of which is incorporated herein by reference.

EXAMPLES

Example I

The synthesis of N-alpha-Ac-Trp-Arg-Tyr-NH₂, is conducted in a
15 stepwise manner on a methylbenzhydrylamine hydrochloride resin, such as available
from Bachem, Inc., having a substitution range of about 0.1 to 0.5 mmoles/g resin.
The synthesis is performed on an automatic Applied Biosystems, Model 430A,
peptide synthesizer using the program supplied by the manufacturer. Briefly, MBHA
resin is placed in the reaction vessel of the peptide synthesizer, and the amino acids,
20 Boc-Tyr(2BrZ)-OH, Boc-Arg(Tos)-OH and Boc-Trp(CHO)-OH, are sequentially
coupled using preformed HOBt ester (2.0 mmol) according to the program provided
by the ABI 430A synthesizer. At the end of the synthesis, alpha-amino group is
acetylated with acetic anhydride and the in-CHO group removed by stirring with 20%

piperidine in DMF for 2 hours. The peptide is detached from the resin by treating with HF containing 5% p. cresol for about 1 h at about -2°C. HF is evacuated under vacuo and the residue transferred to a fritted funnel with diethyl ether and is washed repeatedly with diethyl ether. The crude peptide extracted with acetic acid (10 ml x 2) is diluted to 10% and lyophilized. The peptide is then purified to homogeneity by reversed phase chromatography on a C-18 semi-preparative column (250 x 10 mm) using the buffer systems 0.1% TFA-H₂O and 60% CH₃CN in 0.1% TFA-H₂O. The peptides are characterized by amino acid and mass spectral analysis.

10 Example II

The peptide N-alpha-Ac-Trp-[CH₂NH]-Arg-Tyr-NH₂ is synthesized using the method as described in Example I. However, Boc-Trp-CHO, (*Synthesis* 676-679, 1983), is coupled to H-Arg(Tos)-Tyr(2br-Z)-MBHA-Resin by reductive alkylation in the presence of 1.0% HOAc and 1.0 equiv. of NaBH₃CN in DMF. The crude peptide obtained by HF cleavage is purified as described earlier. Boc-Arg(Z)2-OH is used to prepare the corresponding aldehyde and synthesize N-alpha-Ac-Trp-Arg-[CH₂NH]-Tyr-NH₂.

Example III

20 A peptide dimer is synthesized using the general method as described in Example I, except that after the synthesis of Trp(CHO)-Arg(Tos)-Tyr(2-Br-Z)-MBHA-Resin, the peptide resin is reacted with half an equivalent N, N-Bis-Boc-Cystine, succinic acid, maleic acid or N₂, N₆-di-Boc-Pim (Pim = 2,6-diaminopimelic

acid), in the presence of 1 equivalent of DCC, HOBt and DIEA until the ninhydrin test is negative. The uncoupled alpha-amino group may be acetylated with acetic anhydride. The peptides are then cleaved and purified as described above.

5 **Example IV.**

To study the feeding patterns of animals treated with the tripeptides, rats with intrahypothalamic cannulae are used. Sprague-Dawley rats weighing about 250-350 g are anesthetized with pentobarbital (45 mg/kg) and 24 ga stainless steel cannulae are surgically implanted into the perifornical hypothalamus at the following stereotaxic coordinates taken from the interaural line: A=6.5, L=0.8, V=-2.0 mm. Investigations are performed two weeks after surgery.

A total of 32 rats are divided into four groups in the initial screening. These include control (artificial cerebral spinal fluid, CSF), T-83 (Ac-Tcc-Arg-Tyr-NH₂), T-84 (N-a-indole-3-acetyl-Arg-Tyr-NH₂), and T-91 (Ac-Trp-Arg-Tic(OH)-NH₂). Artificial CSF (CSF, 0.5 µl) or peptides (0.5-20.0 µg) in CSF are injected via the cannulae directly into the hypothalamus and the food intake of rat chow is monitored for 1, 2 and 4 hour periods. Water is available throughout the experiment. The results are shown in Fig. 1.

20 **Example V.**

A feeding experiment is performed as in Example IV except that the 32 rats are divided into four groups which include control (artificial cerebral spinal fluid,

CSF), T-45 (Bis(34/34') {[Cys34]NPY(34-36)}), WX-66 (Ac-D-Trp-Arg-Tyr-NH₂), and WX-126 (Ac-Gln-Arg-Tyr-NH₂). The results are shown in Fig. 2.

Example VI.

- 5 A feeding experiment is performed as in Example IV except that 15 rats are divided into two groups which include control (artificial cerebral spinal fluid, CSF) and T-34 (N-Ac-Trp-Arg-Tyr-NH₂). Food intake of rat chow is monitored for 1,2,4 and 24 hour periods. The results are shown in Fig. 3.

CLAIMS.

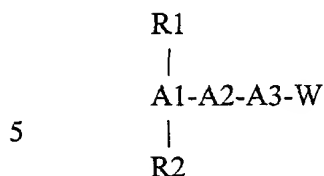
Various features of the invention are emphasized in the claims which follow.

What is claimed is:

5

5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204
2205
2206
2207
2208
2209
2210
2211
2212
2213
2214
2215
2216
2217
2218
2219
2220

1. A compound having the formula:



wherein:

each R1 and R2, independently, is H, C1-C12 alkyl (*e.g.*, methyl), C6-C18 aryl
10 (*e.g.*, phenyl), C1-C18 acyl (*e.g.*, formyl, acetyl, and myristoyl), C7-C18
aralkyl (*e.g.*, benzyl), C7-C18 alkaryl (*e.g.*, p-methylphenyl) or a
dihydrotrigonellinate group;

A1 is a D or L-amino acid selected from the group consisting of Cys, Leu, Dap,
Trp, Gln, a tethered amino acid with an indole ring (*e.g.*, N-Me-Trp), Phe,
15 Hyp, any Trp derivative (*e.g.*, 2 chlorotryptophan, or Tcc); C α Me-Trp, C α Me-
Gln, Des-amino-Trp, Pyr, Bth, Nal, Tcc, Asn, Nva, Abu, Tyr, Tic-OH, Phe,
Tip, and Dip;

A2 is a D or L-amino acid selected from the group consisting of Cys, Trp, Arg,
N-Me-Arg, C α Me-Arg, Orn, Cit, hArg(R)₂ [where R is selected from the
20 group consisting of hydrogen, alkyl, aryl, aralkyl, or alkylaryl], Lys- ϵ -NH-R
[where R is selected from the group consisting of hydrogen, alkyl, aryl,
aralkyl, or alkylaryl];

A3 is a D or L-amino acid selected from the group consisting of Glu, N-Me-Tyr,
C α Me-Tyr, Tic-OH, Tic, Dip, Trp, Phe, des-carboxylic-Tyr (tyramine), and
25 Tyr-(R) [where R is hydrogen or a lipophilic group, *e.g.*, myristoyl,
cholesteryl, t.Bu, *etc.*];

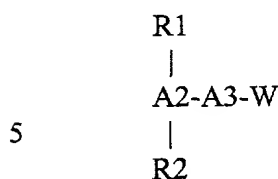
W is -OH, -N-R₃R₄, or OR₅ (where R₃, R₄, and R₅ , independently, is H, C₁-C₁₂ alkyl (*e.g.*, methyl), C₆-C₁₈ aryl (*e.g.*, phenyl), C₁-C₁₂ acyl (*e.g.*, formyl, acetyl, and myristoyl), C₇-C₁₈ aralkyl (*e.g.*, benzyl), or C₇-C₁₈ alkaryl (*e.g.*, p-methylphenyl); or a pharmaceutically acceptable salt thereof;

5 and

each bond between two amino acids or amino acid derivatives, represented by a dash (“-”), can be either a peptide bond or a pseudopeptide bond or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein said compound has a formula selected from the group consisting of N- α -Ac-Trp-Arg-Tyr-NH₂, Ac-Gln-Arg-Tyr-NH₂, Ac-Tcc-Arg-Tyr-NH₂, Ac-Trp-Arg-Tic(OH)-NH₂, and Ac-Tcc-Arg-Tic(OH)-NH₂.

3. A compound having the formula:



wherein:

each R1 and R2, independently, is H, C1-C12 alkyl (*e.g.*, methyl), C6-C18 aryl
10 (*e.g.*, phenyl), C1-C18 acyl (*e.g.*, formyl, acetyl, and myristoyl), C7-C18
aralkyl (*e.g.*, benzyl), C7-C18 alkaryl (*e.g.*, p-methylphenyl) or a
dihydrotrigonellinate group;

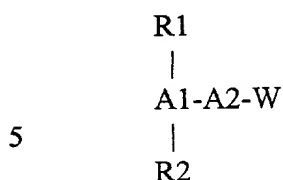
A2 is a D or L-amino acid selected from the group consisting of Cys, Trp, Arg,
N-Me-Arg, C α Me-Arg, Orn, Cit, hArg(R)₂ [where R is selected from the
15 group consisting of hydrogen, alkyl, aryl, aralkyl, or alkylaryl], Lys- ϵ -NH-R
[where R is selected from the group consisting of hydrogen, alkyl, aryl,
aralkyl, or alkylaryl];

A3 is a D or L-amino acid selected from the group consisting of Glu, N-Me-Tyr,
C α Me-Tyr, Tic-OH, Tic, Dip, Trp, Phe, des-carboxylic-Tyr (tyramine), and
20 Tyr-(R) [where R is hydrogen or a lipophilic group, *e.g.*, myristoyl,
cholesteryl, t.Bu, *etc.*];

W is -OH, -N-R₃R₄, or OR₅ (where R₃, R₄, and R₅, independently, is H, C1-
C12 alkyl (*e.g.*, methyl), C6-C18 aryl (*e.g.*, phenyl), C1-C12 acyl (*e.g.*,
formyl, acetyl, and myristoyl), C7-C18 aralkyl (*e.g.*, benzyl), or C7-C18
25 alkaryl (*e.g.*, p-methylphenyl); or a pharmaceutically acceptable salt thereof;
and

each bond between two amino acids or amino acid derivatives, represented by a dash (“-”), can be either a peptide bond or a pseudopeptide bond or a pharmaceutically acceptable salt thereof.

4. A compound having the formula:



wherein:

10 each R1 and R2, independently, is H, C1-C12 alkyl (*e.g.*, methyl), C6-C18 aryl (*e.g.*, phenyl), C1-C18 acyl (*e.g.*, formyl, acetyl, and myristoyl), C7-C18 aralkyl (*e.g.*, benzyl), C7-C18 alkaryl (*e.g.*, p-methylphenyl) or a dihydrotrigonellinate group;

15 A1 is a D or L-amino acid selected from the group consisting of Cys, Leu, Dap, Trp, Gln, a tethered amino acid with an indole ring (*e.g.*, N-Me-Trp), Phe, Hyp, any Trp derivative (*e.g.*, 2 chlorotryptophan, or Tcc); C α Me-Trp, C α Me-Gln, Des-amino-Trp, Pyr, Bth, Nal, Tcc, Asn, Nva, Abu, Tyr, Tic-OH, Phe, Tip, and Dip;

20 A2 is a D or L-amino acid selected from the group consisting of Cys, Trp, Arg, N-Me-Arg, C α Me-Arg, Orn, Cit, hArg(R)₂ [where R is selected from the group consisting of hydrogen, alkyl, aryl, aralkyl, or alkylaryl], Lys- ϵ -NH-R [where R is selected from the group consisting of hydrogen, alkyl, aryl, aralkyl, or alkylaryl];

25 W is -OH, -N-R₃R₄, or OR₅ (where R₃, R₄, and R₅, independently, is H, C1-C12 alkyl (*e.g.*, methyl), C6-C18 aryl (*e.g.*, phenyl), C1-C12 acyl (*e.g.*, formyl, acetyl, and myristoyl), C7-C18 aralkyl (*e.g.*, benzyl), or C7-C18

alkaryl (*e.g.*, *p*-methylphenyl); or a pharmaceutically acceptable salt thereof;
and

each bond between two amino acids or amino acid derivatives, represented by a

dash (“-”), can be either a peptide bond or a pseudopeptide bond or a

5 pharmaceutically acceptable salt thereof.

5. The compound of claim 3, wherein said compound has the formula Ac-Arg-Tyr-NH₂.

6. The compound of claim 4, wherein said compound has the formula Ac-Trp-Arg-NH₂.

7. A compound according to claim 1 further having a formula selected from the group consisting of Cyclo-[-A1-A2-], Cyclo-[-A1-A2-A3-], Cyclo-[-A1-A2-A3-A1-A2-A3-], and Cyclo-[-A1-A2-A3-A3-A2-A1-].

8. The compound of claim 7, wherein said compound is selected from the group consisting of Cyclo (30/34)[Leu 30, Trp 32, Glu 34,]NPY (30-36)-NH₂, Cyclo (30/34)[Dap 30, Trp 32, Glu 34,]NPY (30-36)-NH₂, and N- α -Ac-Cyclo(29/34)[D-Cys 29,34, Trp32]NPY(29-36)-NH₂.

9. A compound according to claim 1 further having the formula:

Ac-[A1-A2-A3]_n-NH₂, wherein n = 1, 2, or 3.

10. The compound of claim 9, wherein, said compound is a dimer of the compounds of claim 1
11. The compound of claim 10, wherein said dimer is prepared by dimerizing the compound with dicarboxylic acids (*e.g.*, succinic acid), cystine, or diaminodicarboxylic acid (*e.g.*, 2,6-diaminopimelic acid).
12. The compound of claim 1, wherein said compound is conjugated to a carrier selected from the group consisting of cationized albumin and polylysine.
13. The compound of claim 1, wherein the said bond between two amino acids or amino acid derivatives is selected from the group consisting of C(O)NH, CH₂NH, CH₂-S, CH₂-O, CH₂-CH₂, CH₂-CO, and CH₂ CH₂.
14. The compound of claim 13, wherein a pseudopeptide bond is positioned between A1 and A2.
15. The compound of claim 14, wherein a pseudopeptide bond is positioned between A2 and A3.

16. The compound of claim 3, wherein the said bond between two amino acids or amino acid derivatives is selected from the group consisting of C(O)NH, CH₂NH, CH₂-S, CH₂-O, CH₂-CH₂, CH₂-CO, and CH₂ CH₂).
17. The compound of claim 4, wherein the said bond between two amino acids or amino acid derivatives is selected from the group consisting of C(O)NH, CH₂NH, CH₂-S, CH₂-O, CH₂-CH₂, CH₂-CO, and CH₂ CH₂).
18. A therapeutic composition capable of controlling an NPY mediated physiological response comprising a therapeutically effective amount of the compound of claim 1, claim 3, claim 4, claim 7 or claim 9 together with a pharmaceutically acceptable carrier substance.
19. The composition of claim 18, wherein said composition is in the form of a pill, tablet, or capsule for oral administration to a subject in need of said compound.
20. The composition of claim 18, wherein said composition is in the form of a liquid for oral administration to a subject in need of said compound.
21. The composition of claim 18, wherein said composition being is in the form of a liquid for nasal administration as drops or spray to a subject in need of said composition.

22. The composition of claim 18, wherein said composition is in the form of a liquid for intravenous, subcutaneous, parenteral, or intraperitoneal administration to a subject in need of said composition.
23. The composition of claim 18, wherein said composition is in the form of a biodegradable sustained- release composition for intramuscular administration to a subject in need of said composition.
24. The composition of claim 18, wherein said composition includes a lipophilic salt and is suitable for administration in the form of an oil emulsion or dispersion to a subject in need of said composition.
25. A method for suppressing an NPY mediated physiological response in a subject comprising administering to said subject a compound of claim 1, claim 3, claim 4, claim 7 or claim 9.
26. The method of claim 25, wherein said administration lowers the blood pressure of said subject.
27. The method of claim 25, wherein said administration suppresses the appetite of said subject.

28. The method of claim 25, wherein said administration augments the libido of said subject.
29. The method of claim 25, wherein said administration stimulates cardiovascular function of said subject.
30. The method of claim 25, wherein said administration modulates the circadian rhythm of said subject.
31. A method of suppressing an NPY mediated physiological response in a tissue other than the heart in a subject comprising administering to said subject a compound of claim 1, claim 3, claim 4, claim 7 or claim 9.
32. The method of claim 31, wherein said compound suppresses the activity of the NPY receptor.
33. The method of claim 32, wherein said compound suppresses the activity of the NPY receptor.
34. A method of suppressing a NPY receptor mediated physiological response in the hypothalamus of a subject comprising administering to said subject the compound of claim 1.

35. A method of suppressing the blood pressure of a subject experiencing hypertension which comprises administering to said subject the compound of claim 1.
36. A method of suppressing a NPY receptor mediated physiological response in the cardiovascular system of a subject comprising administering to said subject the compound of claim 1.
37. A method for stimulating an NPY mediated physiological response in a subject comprising administering to said subject a compound of claim 1, claim 3, claim 4, claim 7 or claim 9.
38. The method of claim 37, wherein said administration increases the blood pressure of said subject.
39. The method of claim 38, wherein said administration increases the appetite of said subject.

COMPOUNDS FOR CONTROL OF APPETITE, BLOOD PRESSURE, CARDIOVASCULAR RESPONSE, LIBIDO, AND CIRCADIAN RHYTHM

**Ambikaipakan Balasubramaniam
William T. Chance**

Background of the Invention

This invention relates generally to dipeptides and tripeptides and to methods for pharmaceutical treatment of mammals using analogs of such dipeptides and tripeptides. More specifically, the invention relates to tripeptides and their analogs, to pharmaceutical compositions containing such dipeptides and tripeptides and to methods of treatment of mammals using such dipeptides and tripeptides. In addition, the invention relates to methods of treatment of mammals using such dipeptides and tripeptides for control of appetite, blood pressure, cardiovascular response, libido, and circadian rhythm.

Figure 1.

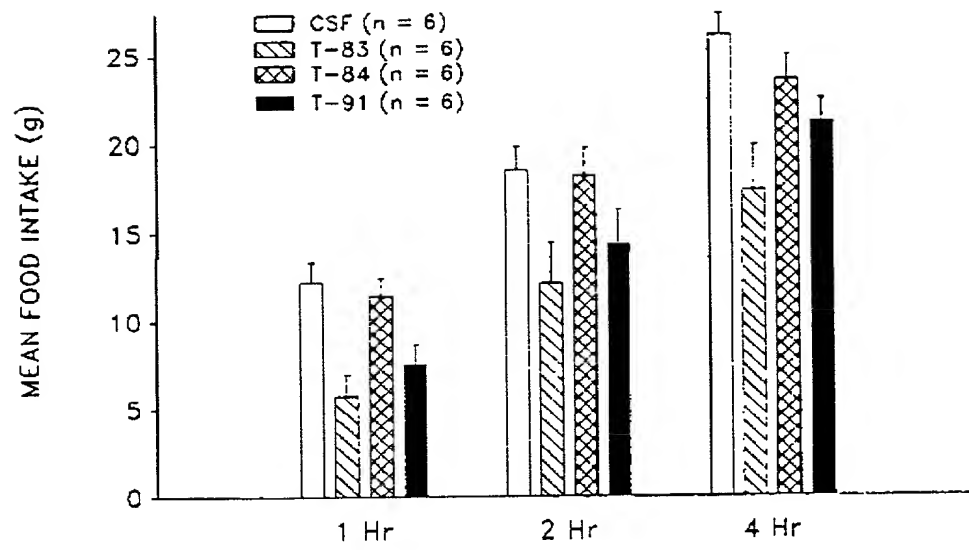


Figure 2.

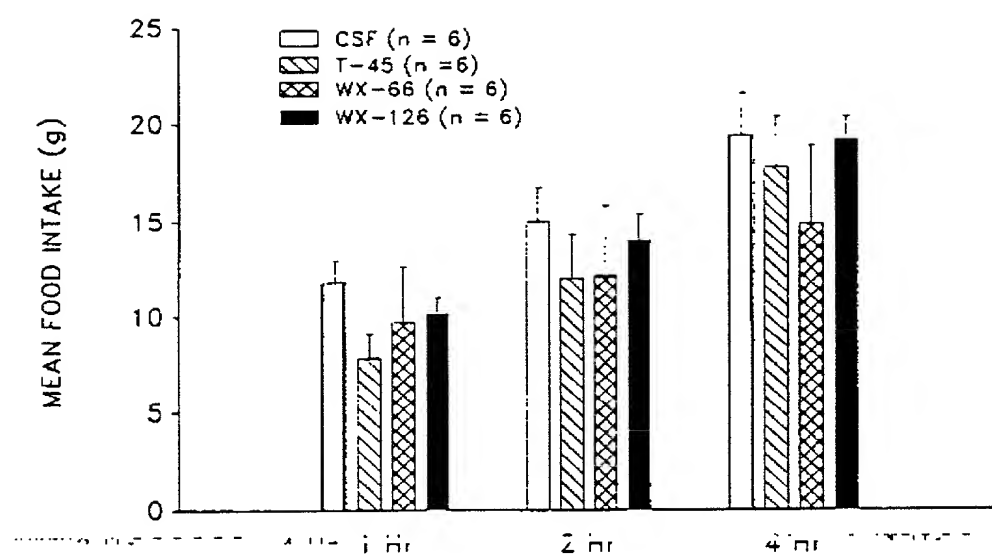
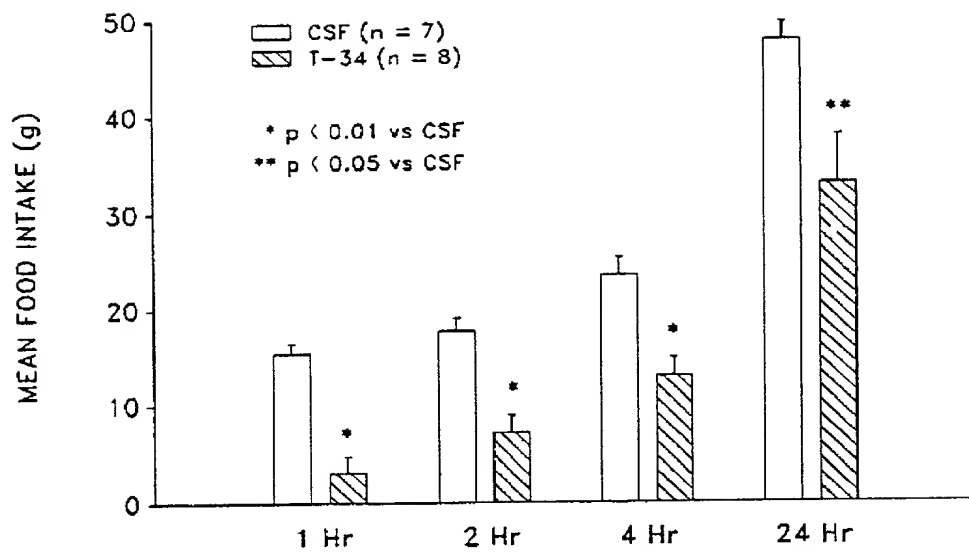


Figure 3.



DECLARATION, POWER OF ATTORNEY, AND PETITION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**COMPOUNDS FOR CONTROL OF APPETITE, BLOOD PRESSURE,
CARDIOVASCULAR RESPONSE, LIBIDO, AND CIRCADIAN RHYTHM**

the specification of which (check one below):

- ☒ is attached hereto.
- ☐ was filed on ____ as Application Serial No. ____ or Express Mail No. ____, and was amended on ____ (if applicable).
- ☐ was filed on ____ as PCT International Application No. ____ , and as amended under PCT Article 19 on ____ (if any).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

Priority Claimed?

_____	_____	_____	() Yes () No
(Number)	(Country)	Day/Month/Year Filed	
_____	_____	_____	() Yes () No
(Number)	(Country)	Day/Month/Year Filed	
_____	_____	_____	() Yes () No
(Number)	(Country)	Day/Month/Year Filed	

I hereby claim the benefit under Title 35, United States Code, §120 and/or §119(e) of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application and the national or PCT international filing date of this application.

<u>09,449,914</u>	<u>December 2, 1999</u>	<u>Pending</u>
(Serial No.)	(Filing Date)	(Status: Patented, Pending, or Abandoned)
<u>08,907,403</u>	<u>August 7, 1997</u>	<u>Patented</u>
(Serial No.)	(Filing Date)	(Status: Patented, Pending, or Abandoned)
_____	_____	_____
(Serial No.)	(Filing Date)	(Status: Patented, Pending, or Abandoned)

I hereby appoint John D. Poffenberger (R. No. 20,245), Bruce Tittel (R. No. 22,324), Donald F. Frei (R. No. 21,190), David J. Josephic (R. No. 22,849), A. Ralph Navaro, Jr. (R. No. 23,050), David S. Stallard (R. No. 25,930), J. Robert Chambers (R. No. 25,448), Gregory J. Lunn (R. No. 29,945), Kurt L. Grossman (R. No. 29,799), Clement H. Luken, Jr. (R. No. 32,742), Thomas J. Burger (R. No. 32,662), Gregory F. Ahrens (R. No. 32,957), Wayne L. Jacobs (R. No. 35,553), Kurt A. Summe (R. No. 36,023), Kevin G. Rooney (R. No. 36,330), Keith R. Haupt (R. No. 37,638), Theodore R. Remaklus (R. No. 38,754), Thomas W. Humphrey (R. No. 34,353), Joseph R. Jordan (R. No. 25,686), C. Richard Eby (R. No. 25,854), David E. Pritchard (R. No. 38,273), David H. Brinkman (R. No. 40,532), J. Dwight Poffenberger, Jr. (R. No. 35,324), Beverly A. Lyman (R. No. 41,961), A. Ralph Navaro III (R. No. 46,207), Scott A. Stinebruner (R. No. 38,323), Kristi L. Davidson (R. No. 44,643), P. Andrew Blatt (R. No. 44,540), David E. Jefferies (R. No. P-46,800), David E. Franklin (R. No. 39,194), Herbert C. Brinkman (R. No. 16,955), all

of Wood, Herron & Evans, L.L.P., 2700 Carew Tower, 441 Vine Street, Cincinnati, OH 45202-2917, telephone no. (513) 241-2324, my attorneys, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Address all correspondence and telephone calls to

Gregory J. Lunn
Wood, Herron & Evans, L.L.P.
2700 Carew Tower
441 Vine Street
Cincinnati, OH 45202-2917
Telephone (513) 241-2324

Wherefore I pray that Letters Patent be granted to me for the invention or discovery described and claimed in the foregoing specification and claims, and I hereby subscribe my name to the foregoing specification and claims, declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of Inventor Ambikaipakan Balasubramaniam

Inventor's Signature Ambikaipakan Balasubramaniam Date 07/05/00

Residence City/State 5100 Autumnwood Dr., Cincinnati, Ohio Citizenship U.S.A.

Post Office Address 5100 Autumnwood Dr., Cincinnati, OH 45242

Full name of Inventor William T. Chance

Inventor's Signature William T. Chance Date 07/05/00

Residence City/State 854 Gorham Pl., Withamsville, Ohio Citizenship U.S.A.

Post Office Address 854 Gorham Pl., Withamsville, OH 45245